# Fructooligosaccharides and *Lactobacillus acidophilus* Modify Gut Microbial Populations, Total Tract Nutrient Digestibilities and Fecal Protein Catabolite Concentrations in Healthy Adult Dogs[1]

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ABSTRACT The objective of this research was to determine whether fructooligosaccharides (FOS) and (or) *Lactobacillus acidophilus* (LAC) affected concentrations of gut microbial populations, fermentative end products and nutrient digestibilities in healthy adult dogs. Two experiments were performed using 40 adult dogs (20 dogs/experiment). Dogs in each experiment were randomly assigned to one of 4 treatments. Twice daily, treatments were given orally via gelatin capsules: *1*) 2 g sucrose + 80 mg cellulose; *2*) 2 g FOS + 80 mg cellulose; *3*) 2 g sucrose + $1 \times 10^9$ colony forming units (cfu) LAC; or *4*) 2 g FOS + $1 \times 10^9$ cfu LAC. Data were analyzed by the General Linear Models procedure of SAS. In Experiment 1, FOS resulted in lower ($P = 0.08$) *Clostridium perfringens* and greater fecal butyrate ($P = 0.06$) and lactate ($P < 0.05$) concentrations. In Experiment 2, FOS supplementation increased ($P < 0.05$) bifidobacteria, increased lactobacilli ($P = 0.08$), increased fecal lactate ($P = 0.06$) and butyrate ($P < 0.05$), and decreased ($P < 0.05$) fecal ammonia, isobutyrate, isovalerate and total branched-chain fatty acid concentrations. Dogs fed LAC had the highest fecal concentrations of hydrogen sulfide and methanethiol in Experiment 1 and dimethyl sulfide in Experiment 2, whereas dogs fed FOS had the lowest concentrations of these compounds. Overall, FOS appeared to enhance indices of gut health by positively altering gut microbial ecology and fecal protein catabolites, whereas LAC was more effective when fed in combination with FOS rather than fed alone. J. Nutr. 132: 3721–3731, 2002.

KEY WORDS: • *dogs* • *prebiotics* • *probiotics* • *fructooligosaccharides* • Lactobacillus

It is generally accepted that a nutritionally balanced diet and a proper microbial ecology are required for a healthy gut. Improving gut health through the use of prebiotics, probiotics or the combination of the two (synbiotics) has become an area of research activity in both human and animal nutrition. A probiotic is a live microbial food supplement that beneficially affects the host by improving its intestinal microbial balance (1). Gibson and Roberfroid (2) introduced the concept of prebiotics to alter microbial populations and, consequently, improve host health. By definition, a prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health (2). Survivability, colonization and the beneficial effects of feeding an exogenous probiotic may be enhanced and extended by simultaneous administration of a prebiotic that the probiotic can utilize in the intestinal tract (3). Synbiotics, the combination of a probiotic and prebiotic (2,4), may have potential for improving gut health.

The most common prebiotics studied are fructans, also referred to as fructooligosaccharides (FOS).[3] Although the term FOS is often used to refer to all nondigestible oligosaccharides composed of fructose and glucose units, it refers specifically to short chains (~3–6 units) of fructose units bound by $\beta$-(2–1) linkages that are attached to a terminal glucose unit. Because the $\beta$-(2–1) fructose linkages are resistant to mammalian enzymes, fructans reach the colon and serve as a source of highly digestible substrate for colonic bacteria. Lactate-producing bacterial genera, such as *Lactobacillus* and *Bifidobacterium*, are commonly used in probiotic products because of their health-promoting properties in the gut (5).

Although a large body of literature exists regarding the effects of prebiotics and probiotics on human health (6,7), only a small amount of research exists using the canine. The focus of most experiments using dogs has been narrow, with most groups focusing on microbial populations and fecal con-

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[3] Abbreviations used: AA, amino acids; BCFA, branched-chain fatty acids; cfu, colony-forming units; CP, crude protein; DM, dry matter; FOS, fructooligosaccharides; LAC, *Lactobacillus acidophilus*; OM, organic matter; SCFA, short-chain fatty acids.


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sistency. The effects of probiotics and synbiotics on gut health indices for dogs have been virtually ignored. The objectives of this research were to determine whether dietary supplementation with FOS and (or) *Lactobacillus acidophilus* (LAC) affects colonic microbial populations, fecal concentrations of fermentative end products, and total tract nutrient digestibilities using healthy adult dogs.

## MATERIALS AND METHODS

***Animals and diets.*** Healthy male and female adult dogs ($n = 40$; Pointers) were used in two experiments. In each experiment, 20 dogs were randomly assigned to one of four treatments (5 dogs/treatment). Dogs were individually housed in indoor/outdoor kennels at a commercial kennel. The indoor section was 1.3 × 1.7 m and the outdoor section was 1.3 × 3.3 m. Animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before initiation of the experiment. Dogs had free access to water and were fed 300 g diet twice daily. Fructooligosaccharide-free ingredients were used, with pregelatinized cornstarch, meat and bone meal, poultry by-product meal and poultry fat constituting the main ingredients of the dry, extruded, kibble diet (**Table 1**). The formulation resulted in a diet containing 23.8% crude protein (CP), 18.4% fat, 12.9% ash, and 6.0% total dietary fiber.

At each feeding, treatments were administered orally via gelatin capsules: *1*) 2 g sucrose + 80 mg microcrystalline cellulose (control); *2*) 2 g FOS + 80 mg microcrystalline cellulose; *3*) 2 g sucrose + $1 \times 10^9$ colony forming units (cfu) of LAC; and *4*) 2 g FOS + $1 \times 10^9$ cfu of LAC. The short-chain FOS (NutraFlora, 95% FOS powder) was obtained from GTC Nutrition (Golden, CO). Hard gelatin capsules were filled with 80 mg freeze dried *Lactobacillus acidophilus* NCFM (Rhodi, Madison, WI) or 80 mg microcrystalline cellulose (Avicel, FMC, Philadelphia, PA) only. For the LAC capsules, the minimum allowable count of LAC was $1 \times 10^9$ cfu/capsule. Cellulose and LAC capsules were coated with hydroxypropyl methylcellulose phthalate (HP555, Shin-Etsu, Tokyo, Japan) by spraying on a fluidized bed of capsules, resulting in a relatively nonporous, low-surface-area product that is resistant to stomach acid. The doses of FOS (4 g FOS/d) and LAC ($2 \times 10^9$ cfu LAC/d) used for this experiment were based on the effective doses reported in the literature (8–10) and data from previous experiments performed in our laboratory (11).

**TABLE 1**

*Ingredient and chemical composition of the diet fed to dogs in both experiments*[1]

| Ingredient | *g/kg* |
|---|---|
| Pregelatinized cornstarch[2] | 352.5 |
| Meat and bone meal | 271.1 |
| Poultry by-product meal | 158.9 |
| Poultry fat | 111.0 |
| Beet pulp | 39.0 |
| Dehydrated egg | 21.0 |
| Chicken digest | 20.0 |
| Fish oil | 12.0 |
| Sodium chloride | 6.5 |
| Potassium chloride | 4.3 |
| Choline chloride | 1.3 |
| Vitamin premix | 1.2 |
| Mineral premix | 1.2 |
| Analyzed composition | |
| Dry matter, *g/100 g* | 94.0 |
| | *g/100 g dry matter* |
| Organic matter[3] | 87.1 |
| Ash | 12.9 |
| Crude protein | 23.8 |
| Fat | 18.4 |
| Total dietary fiber | 6.0 |
| Gross energy, *kJ/g* | 20.5 |

[1] Provided per kg of diet: vitamin A, 4.31 mg; vitamin D, 25.5 μg; vitamin E, 70.61 mg; vitamin K, 0.63 mg; thiamin, 7.70 mg; riboflavin, 10.92 mg; pantothenic acid, 15.75 mg; niacin, 99.61 mg; pyridoxine, 8.34 mg; choline, 1,937.24 mg; biotin, 189.48 μg; folic acid, 1,204.30 μg; vitamin B-12, 114.72 μg; manganese, 20.14 mg; iron, 369.20 mg; copper, 16.04 mg; cobalt, 2.57 mg; zinc, 173.87 mg; iodine, 4.51 mg; selenium, 0.17 mg.

[2] Nat'l 1215 starch (Chicago Sweeteners, Des Plaines, IL).

[3] Organic matter = dry matter − ash.

***Sample collection.*** Each experiment consisted of a 23-d adaptation period followed by a 5-d collection period. During the collection phase, total and fresh fecal samples were collected. A fresh fecal sample was obtained between d 24 and 28 for bacterial enumeration and analysis of fermentation end products [ammonia, biogenic and monogenic amines, branched-chain fatty acids (BCFA), indoles, lactate, phenols, short-chain fatty acids (SCFA)], pH, dry matter (DM) % and organic matter (OM) %.

Total feces excreted during the collection phase of each period were removed from the floor of the pen, weighed, composited, and frozen at –20°C. All fecal samples from d 22 to 28 were scored according to the following system: 1 = hard, dry pellets; small, hard mass; 2 = hard, formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool; 4 = soft, unformed stool; assumes shape of container; 5 = watery; liquid that can be poured.

***Sample handling.*** Feces and diets were dried at 55°C in a forced-air oven. After drying, diets and fecal samples were ground through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ).

Fresh fecal samples were collected within 15 min of defecation and an aliquot was immediately transferred to a preweighed Carey-Blair transport media container (Meridian Diagnostic, Cincinnati, OH) for subsequent bacterial enumeration (total anaerobes, total aerobes, *Bifidobacterium*, *Lactobacillus*, *Clostridium perfringens*, and *Escherichia coli*). Additional aliquots were collected for pH measurement and determination of fermentative end products.

One aliquot was used to measure fecal volatile sulfur compound concentrations. This aliquot was placed into a test tube, flushed with $CO_2$, maintained at 37°C and immediately transported to the laboratory. Fresh sample (5 g) was combined with 15 mL PBS and blended under a steady stream of nitrogen. The fecal mixture (5 mL) was then placed in a 60 mL polypropylene syringe. After purging the syringe and contents with nitrogen three times, 15 mL of nitrogen was added to the syringe. The syringe then was sealed with a rubber tubing septa (Sigma-Aldrich Z10,072–2; St. Louis, MO). Syringes were incubated at 39°C for 2, 4 and 24 h. One syringe was prepared for each time point for each sample. At the appropriate incubation time, the syringe was removed from the incubator. After the total volume of gas produced in the syringe was measured, 250 μL of gas were analyzed by gas chromatography.

A second aliquot (~10 g; used to measure SCFA, BCFA, ammonia and lactate) was acidified with 10 mL HCl and stored at –20°C until analysis. Additional aliquots were stored at –20°C until biogenic and monogenic amine, indole and phenol concentrations could be measured.

***Chemical analyses.*** Diets and fecal samples were analyzed for DM, OM, and ash using AOAC (12) methods. Crude protein was calculated from Kjeldahl N values (12). Total lipid content was determined by acid hydrolysis followed by ether extraction according to American Association of Cereal Chemists (13) and Budde (14). Total dietary fiber concentration was determined according to Prosky et al. (15,16). Ammonia concentrations were measured according to the method of Chaney and Marbach (17). Chromium concentration was analyzed according to Williams et al. (18) using an atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, Norwalk, CT). SCFA concentrations were determined via gas chromatography according to Erwin et al. (19). Briefly, concentrations of acetate, propionate, butyrate, valerate, isovalerate and isobutyrate were determined in the supernate of acidified fecal aliquots using a Hewlett-Packard 5890A Series II gas chromatograph (Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% $H_3PO_4$ on 80/100+ mesh Chromosorb WAW (Supelco, Bellefonte,

PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven temperature, detector temperature and injector temperature were 125, 175, and 180°C, respectively. Lactate concentrations were measured by the spectrophotometric method described by Barker and Summerson (20). Phenol and indole concentrations were determined via gas chromatography according to Flickinger et al. (21). Biogenic amine concentrations were determined via HPLC according to Flickinger et al. (21).

Volatile sulfur compounds were measured using gas chromatography according to Suarez et al. (22). Briefly, concentrations of hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide were determined using a Hewlett-Packard 5890 gas chromatograph, Sievers sulfur chemiluminescence detector (Model 355 SCD, Ionics Instrument Business Group, Boulder, CO), and WCOT fused silica column (50 m × 0.32 i.d.) made by Chrompack (Middelburg, The Netherlands). Helium was the carrier gas with a flow rate of 100 mL/min.

Microbial populations were determined by serial dilution ($10^{-1}$ to $10^{-7}$) of fecal samples in anaerobic diluent before inoculation onto petri dishes of sterile agar as described by Bryant and Burkey (23). Total anaerobe and total aerobe agars were prepared according to Bryant and Robinson (24) and Mackie et al. (25). The selective media for bifidobacteria (BIM-25) was prepared using reinforced clostridial agar (BBL Microbiology Systems, Cockeyville, MD) according to Muñoa and Pares (26). Lactobacilli were grown on Rogosa SL agar (Difco Laboratories, Detroit, MI). *E. coli* were grown on eosin methylene blue agar (Difco Laboratories, Detroit, MI). Agars used to grow C. *perfringens* were prepared according to the FDA Bacteriological Analytical Manual (27). Plates for total anaerobes, *Bifidobacterium*, *Lactobacillus* and C. *perfringens* were incubated anaerobically (73% N:20% $CO_2$:7% $H_2$) at 37°C. Total aerobes and *E. coli* were incubated aerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. Colony forming units were defined as being distinct colonies measuring at least 1 mm in diameter.

***Calculations.*** Dry matter (g/d) recovered as feces was calculated by dividing the Cr intake (mg/d) by fecal Cr concentrations (mg Cr/g feces). Fecal nutrient flows were calculated by multiplying DM flow by the concentration of the nutrient in the fecal DM. Total tract nutrient digestibilities were calculated as nutrient intake (g/d) minus the fecal nutrient flow (output, g/d), divided by nutrient intake (g/d).

***Statistical analyses.*** All data, except that of volatile sulfur compounds, were analyzed by the General Linear Models procedure of SAS (SAS Institute, Cary, NC). A 2 × 2 factorial arrangement of treatments was used in each experiment. After being log-transformed, volatile sulfur compound data were analyzed using the Proc Mixed procedure of SAS. The Ante-Dependence structure was used to analyze the repeated-measures data points collected for sulfur compounds. A probability of $P < 0.05$ was accepted as being significant although mean differences with $P < 0.15$ were accepted as trends and results are discussed accordingly.

## RESULTS

***Animals.*** In general, dogs in Experiment 1 were older and weighed more than those in Experiment 2 (**Table 2**). Dogs in Experiment 1 had a mean body weight of 23.0 kg (range = 18.7–31.9 kg) and mean age of 6.3 y (range = 0.9–10.8 y). Dogs in Experiment 2 had a mean body weight of 21.2 kg (range = 16.7–29.1 kg) and mean age of 2.2 y (range = 0.9–5.9 y).

***Food intake and fecal characteristics.*** In Experiment 1, dogs fed LAC had lower ($P < 0.05$) food intake and fecal output (**Table 3**) than dogs consuming cellulose. Fecal DM %, pH and scores were not different among treatments in Experiment 1 (Table 3). In Experiment 2, dogs fed FOS tended ($P = 0.13$) to have lower food intake than dogs fed sucrose. Lower food intake resulted in lower (as-is, $P = 0.06$; DM basis, $P = 0.05$) fecal output by FOS-supplemented dogs. When FOS and LAC were fed alone, they did not affect fecal pH or score in Experiment 2. However, significant ($P < 0.05$) interaction effects were observed. Fecal pH and scores were greater for dogs fed the combination of FOS + LAC compared with dogs fed FOS or LAC alone. Fecal DM percentage was not affected by treatment in Experiment 2.

***Microbial populations.*** The probiotic, LAC, did not affect ($P > 0.05$) microbial populations in Experiment 1 (**Table 4**). However, dogs supplemented with LAC in Experiment 2 tended to have greater ($P = 0.08$) bifidobacteria concentrations. Fructooligosaccharide supplementation influenced microbial populations in both experiments. In Experiment 1, dogs fed FOS tended to have lower ($P = 0.08$) C. *perfringens* concentrations than dogs consuming sucrose. Dogs fed FOS in Experiment 2 had greater ($P < 0.05$) total aerobe and bifidobacteria concentrations than dogs consuming sucrose. Dogs fed FOS also tended to have greater ($P = 0.08$) lacto-

**TABLE 2**

*Age and body weights (BW) of dogs fed diets with or without fructooligosaccharides (FOS) and (or) Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| Experiment 1 | | | | | | | | |
| Age, y | 5.6 | 4.1 | 8.4 | 6.9 | 1.39 | 0.28 | 0.06 | 0.99 |
| Beginning BW, *kg* | 23.9 | 23.4 | 23.4 | 21.2 | 1.36 | 0.34 | 0.33 | 0.55 |
| Ending BW, *kg* | 24.2 | 23.5 | 23.2 | 21.4 | 1.41 | 0.37 | 0.29 | 0.70 |
| Experiment 2 | | | | | | | | |
| Age, *y* | 2.6 | 2.5 | 1.8 | 1.9 | 0.60 | 0.98 | 0.24 | 0.87 |
| Beginning BW, *kg* | 22.3 | 21.1 | 22.0 | 19.3 | 1.33 | 0.16 | 0.47 | 0.58 |
| Ending BW, *kg* | 23.2 | 22.0 | 23.2 | 21.8 | 1.18 | 0.29 | 0.93 | 0.91 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.

## TABLE 3

*Food intake and fecal characteristics of dogs fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| Experiment 1 | | | | | | | | |
| Food intake (as-is), *g/d* | 561 | 507 | 446 | 465 | 81.57 | 0.64 | 0.05 | 0.33 |
| Fecal output (as-is), *g/d* | 248 | 241 | 185 | 174 | 22.96 | 0.69 | 0.01 | 0.95 |
| Fecal DM, *g/100 g* | 39.4 | 40.7 | 41.4 | 41.2 | 1.21 | 0.65 | 0.34 | 0.55 |
| Fecal output (DMB), *g/d* | 98 | 98 | 76 | 71 | 8.90 | 0.78 | 0.01 | 0.80 |
| Fecal pH | 6.50 | 6.33 | 6.37 | 6.23 | 0.26 | 0.55 | 0.65 | 0.95 |
| Fecal score[7] | 2.18 | 1.96 | 1.90 | 2.04 | 0.20 | 0.84 | 0.62 | 0.37 |
| Experiment 2 | | | | | | | | |
| Food intake (as-is), *g/d* | 600 | 561 | 579 | 581 | 11.30 | 0.13 | 0.99 | 0.09 |
| Fecal output (as-is), *g/d* | 288 | 232 | 267 | 242 | 20.10 | 0.06 | 0.79 | 0.44 |
| Fecal DM, *g/100 g* | 39.1 | 40.1 | 40.2 | 39.2 | 0.68 | 0.94 | 0.90 | 0.16 |
| Fecal output (DMB), *g/d* | 113 | 93 | 107 | 95 | 7.55 | 0.05 | 0.81 | 0.64 |
| Fecal pH | 6.53 | 6.30 | 6.16 | 6.76 | 0.13 | 0.20 | 0.73 | 0.01 |
| Fecal score[7] | 2.38 | 2.10 | 2.07 | 2.48 | 0.11 | 0.59 | 0.79 | 0.01 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.
[7] Fecal scoring system: 1 = hard, dry pellets; small, hard mass; 2 = hard, formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool; 4 = soft, unformed stool; assumes shape of container; 5 = watery; liquid that can be poured.

bacilli concentrations. In Experiment 1, dogs fed the synbiotic (FOS + LAC) tended to have greater ($P = 0.09$) total anaerobe concentrations than those fed FOS or LAC alone.

***Fecal SCFA and lactate concentrations.*** In Experiment 1, FOS-supplemented dogs had greater fecal butyrate ($P = 0.06$) and lactate ($P < 0.05$) concentrations than dogs fed

## TABLE 4

*Fecal microbial populations for dogs fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| | *cfu $log_{10}$/g fecal DM*[7] | | | | | | | |
| Experiment 1 | | | | | | | | |
| Total anaerobes | 10.70 | 10.46 | 10.47 | 10.67 | 0.12 | 0.85 | 0.93 | 0.09 |
| Total aerobes | 9.44 | 9.45 | 9.07 | 9.75 | 0.24 | 0.17 | 0.89 | 0.18 |
| *Bifidobacterium* | 9.37 | 9.52 | 9.06 | 9.65 | 0.22 | 0.11 | 0.69 | 0.33 |
| *Lactobacillus* | 9.29 | 9.27 | 8.91 | 9.68 | 0.31 | 0.25 | 0.98 | 0.22 |
| *Clostridium perfringens* | 9.90 | 9.62 | 9.81 | 9.63 | 0.12 | 0.08 | 0.75 | 0.67 |
| *Escherichia coli* | 7.30 | 7.20 | 7.09 | 6.18 | 0.43 | 0.26 | 0.17 | 0.36 |
| Experiment 2 | | | | | | | | |
| Total anaerobes | 10.40 | 10.62 | 10.55 | 10.67 | 0.15 | 0.27 | 0.52 | 0.75 |
| Total aerobes | 9.31 | 9.94 | 9.11 | 10.15 | 0.29 | 0.01 | 0.98 | 0.49 |
| *Bifidobacterium* | 9.35 | 9.93 | 9.82 | 10.02 | 0.15 | 0.02 | 0.08 | 0.21 |
| *Lactobacillus* | 9.13 | 9.79 | 8.95 | 9.62 | 0.36 | 0.08 | 0.63 | 0.98 |
| *C. perfringens* | 9.71 | 9.60 | 9.64 | 9.77 | 0.15 | 0.96 | 0.75 | 0.46 |
| *E. coli* | 7.67 | 7.25 | 7.65 | 7.26 | 0.44 | 0.37 | 0.98 | 0.97 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.
[7] cfu, colony-forming units; DM, dry matter.

**TABLE 5**

*Fecal short-chain fatty acid (SCFA) and lactate concentrations for dogs fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | FOS | | LAC | | FOS/LAC | | | *P*-values | | |
| Item | *μmol* | MR[3] | *μmol* | MR | *μmol* | MR | *μmol* | MR | SEM[4] | FOS[5] | LAC[6] | FOS × LAC[7] |
| Experiment 1 | | | | | | | | | | | | |
| Total SCFA[8] | 433.7 | | 514.8 | | 459.9 | | 499.6 | | 37.47 | 0.13 | 0.89 | 0.59 |
| Acetate | 309.4 | 71.3 | 337.0 | 65.5 | 308.1 | 67.0 | 335.2 | 67.1 | 31.21 | 0.39 | 0.96 | 0.99 |
| Propionate | 83.6 | 19.3 | 119.6 | 23.2 | 99.4 | 21.6 | 108.2 | 21.6 | 5.94 | <0.01 | 0.72 | 0.04 |
| Butyrate | 40.8 | 9.4 | 58.2 | 11.3 | 52.5 | 11.4 | 56.3 | 11.3 | 5.24 | 0.06 | 0.37 | 0.21 |
| Lactate | 2.7 | | 41.7 | | 7.6 | | 86.6 | | 23.85 | 0.03 | 0.31 | 0.41 |
| Experiment 2 | | | | | | | | | | | | |
| Total SCFA[8] | 513.8 | | 592.1 | | 558.4 | | 500.9 | | 38.11 | 0.79 | 0.55 | 0.09 |
| Acetate | 371.2 | 72.2 | 414.4 | 70.0 | 401.6 | 71.9 | 357.3 | 71.3 | 32.05 | 0.99 | 0.68 | 0.19 |
| Propionate | 94.4 | 18.4 | 114.3 | 19.3 | 106.7 | 19.1 | 85.8 | 17.1 | 8.91 | 0.96 | 0.38 | 0.04 |
| Butyrate | 48.2 | 9.4 | 63.4 | 10.7 | 50.1 | 9.0 | 57.8 | 11.5 | 5.15 | 0.04 | 0.73 | 0.48 |
| Lactate | 17.3 | | 70.2 | | 5.0 | | 32.8 | | 19.84 | 0.06 | 0.23 | 0.54 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] MR = molar ratios of acetate, propionate, and butyrate.
[4] SEM = pooled SEM.
[5] Main effect of FOS.
[6] Main effect of LAC.
[7] Interaction effect between FOS and LAC.
[8] Total SCFA = acetate + propionate + butyrate.

sucrose (**Table 5**). Similar results were observed in Experiment 2 because greater fecal butyrate ($P < 0.05$) and lactate ($P = 0.06$) concentrations were measured in dogs fed FOS. In Experiment 2, dogs fed the synbiotic had lower ($P < 0.05$) fecal propionate concentrations compared with dogs fed FOS or LAC alone. A similar trend ($P = 0.10$) was observed with total fecal SCFA concentrations. Probiotic supplementation did not influence fecal SCFA and lactate concentrations in this experiment. Molar ratios of SCFA were similar among treatments in both experiments.

***Fecal BCFA and ammonia concentrations.*** Fecal BCFA concentrations, a good indicator of protein catabolism in the large bowel, were affected by FOS supplementation (**Table 6**). No main effects of FOS or LAC supplementation were observed in Experiment 1. However, dogs fed FOS + LAC had lower fecal isobutyrate ($P = 0.07$), isovalerate ($P < 0.05$) and total BCFA ($P < 0.05$) concentrations than dogs fed FOS or LAC alone. In Experiment 2, dogs fed FOS had lower ($P < 0.05$) fecal isobutyrate, isovalerate and total BCFA concentrations than dogs fed sucrose. Fecal ammonia concentrations were similar among treatments in dogs in Experiment 1. Dogs in Experiment 2 fed FOS + LAC had lower ($P < 0.05$) fecal ammonia concentrations than dogs fed FOS or LAC alone.

***Fecal phenol and indole concentrations.*** Dogs fed FOS had lower ($P = 0.05$) total phenol (phenol + *p*-cresol + 4-ethylphenol) concentrations than dogs fed sucrose in Experiment 1 (**Table 7**). Dogs in this experiment fed the FOS + LAC had lower phenol ($P = 0.07$), indole ($P < 0.05$), and total phenol and indole (phenol + *p*-cresol + 4-ethylphenol + indole; $P < 0.05$) concentrations than dogs fed FOS or LAC alone. However, dogs in Experiment 1 fed the synbiotic tended to have greater ($P = 0.10$) 4-ethylphenol concentrations than dogs consuming FOS or LAC alone. This same trend ($P = 0.06$) was observed for 4-ethylphenol concentrations in Experiment 2. Dogs in Experiment 2 fed FOS tended to have lower ($P = 0.07$) indole concentrations than dogs fed sucrose. Total phenol and indole concentrations were not different among treatments in Experiment 2.

***Fecal biogenic and monogenic amine concentrations.*** No main effects of FOS or LAC supplementation on fecal biogenic amine concentrations were observed in Experiment 1 (**Table 8**). However, interaction effects were observed in this experiment. Dogs given FOS + LAC tended to have lower fecal histamine ($P = 0.06$), spermine ($P = 0.08$) and total biogenic amine ($P = 0.09$) concentrations than dogs supplemented with FOS or LAC alone. No differences ($P > 0.05$) in fecal biogenic or monogenic amine concentrations were observed among treatments in Experiment 2 (**Table 9**).

***Fecal volatile sulfur-containing compound concentrations.*** Because only trace amounts of dimethyl disulfide and dimethyl trisulfide were detected in the samples collected, they could not be quantified. Therefore, only hydrogen sulfide, methanethiol and dimethyl sulfide concentration data were analyzed statistically. In Experiment 1, concentrations of hydrogen sulfide, methanethiol and dimethyl sulfide increased ($P < 0.05$) with time of fermentation (**Table 10**). Treatment differences were observed in fecal hydrogen sulfide ($P < 0.05$) and methanethiol ($P = 0.07$) concentrations. However, no treatment differences were observed in fecal dimethyl sulfide concentrations. Hydrogen sulfide and methanethiol concentrations were highest in fecal samples from dogs fed LAC + sucrose.

In general, concentrations of hydrogen sulfide, methanethiol and dimethyl sulfide were greater in samples from dogs in Experiment 2 vs. Experiment 1. In Experiment 2, concentrations of hydrogen sulfide and dimethyl sulfide increased ($P < 0.05$) as time of fermentation increased (**Table 11**). However, time of fermentation did not affect ($P > 0.05$) methanethiol concentrations. Treatment effects were not observed ($P > 0.05$) in hydrogen sulfide and methanethiol concentrations.

## TABLE 6

*Fecal branched-chain fatty acids (BCFA) and ammonia concentrations for dogs fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| | *μmol/g dry matter* | | | | | | | |
| Experiment 1 | | | | | | | | |
| Valerate | 0.66 | 0.94 | 0.65 | 0.77 | 0.13 | 0.14 | 0.47 | 0.53 |
| Isobutyrate | 6.85 | 7.39 | 8.29 | 5.89 | 0.74 | 0.23 | 0.97 | 0.07 |
| Isovalerate | 10.51 | 12.07 | 13.42 | 9.86 | 1.17 | 0.40 | 0.77 | 0.04 |
| Total BCFA[7] | 18.03 | 20.40 | 22.36 | 16.51 | 1.89 | 0.37 | 0.91 | 0.05 |
| Ammonia | 127.27 | 135.88 | 136.27 | 120.78 | 11.90 | 0.78 | 0.80 | 0.33 |
| Experiment 2 | | | | | | | | |
| Valerate | 0.67 | 0.70 | 1.00 | 0.63 | 0.14 | 0.24 | 0.35 | 0.15 |
| Isobutyrate | 7.59 | 6.03 | 7.83 | 5.88 | 0.49 | <0.01 | 0.92 | 0.70 |
| Isovalerate | 12.16 | 9.76 | 11.88 | 9.49 | 0.70 | <0.01 | 0.70 | 1.00 |
| Total BCFA[7] | 20.42 | 16.49 | 20.71 | 16.00 | 1.24 | <0.01 | 0.94 | 0.76 |
| Ammonia | 131.31 | 137.02 | 162.46 | 124.39 | 6.80 | 0.03 | 0.19 | 0.01 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.
[7] Total BCFA = valerate + isobutyrate + isovalerate.

Treatment tended ($P = 0.09$) to affect dimethyl sulfide concentrations in samples from Experiment 2. Similar to the observations with hydrogen sulfide and methanethiol concentrations in Experiment 1, dogs fed SUC + LAC in Experiment 2 had greater concentrations of dimethyl sulfide than did the other treatments.

## TABLE 7

*Fecal phenol and indole concentrations for dogs fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| | *μmol/g dry matter* | | | | | | | |
| Experiment 1 | | | | | | | | |
| Phenol | 0.47 | 0.69 | 1.28 | 0.32 | 0.31 | 0.24 | 0.50 | 0.07 |
| 4-Ethylphenol | 1.21 | 0.53 | 0.47 | 0.64 | 0.24 | 0.31 | 0.22 | 0.10 |
| Indole | 1.22 | 1.27 | 1.61 | 0.61 | 0.25 | 0.07 | 0.59 | 0.05 |
| Total phenols[7] | 1.69 | 1.22 | 1.74 | 0.96 | 0.30 | 0.05 | 0.74 | 0.60 |
| Total indoles and phenols[8] | 2.91 | 2.49 | 3.36 | 1.57 | 0.24 | 0.07 | 0.59 | 0.05 |
| Experiment 2 | | | | | | | | |
| Phenol | 0.43 | 0.51 | 0.67 | 0.33 | 0.20 | 0.52 | 0.87 | 0.32 |
| 4-Ethylphenol | 0.74 | 0.57 | 0.44 | 0.90 | 0.16 | 0.38 | 0.94 | 0.06 |
| Indole | 1.11 | 0.67 | 1.14 | 0.72 | 0.22 | 0.07 | 0.88 | 0.97 |
| Total phenols[7] | 1.18 | 1.07 | 1.10 | 1.23 | 0.27 | 0.97 | 0.88 | 0.67 |
| Total indoles and phenols[8] | 2.29 | 1.75 | 2.24 | 1.95 | 0.40 | 0.32 | 0.85 | 0.76 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.
[7] Total phenols = phenol + *p*-cresol + 4-ethylphenol.
[8] Total indoles and phenols = phenol + *p*-cresol + 4-ethylphenol + indole.

## TABLE 8

*Fecal biogenic and monogenic amine concentrations for dogs of Experiment 1 fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| | *μmol/g dry matter* | | | | | | | |
| Total biogenic amines[7] | 7.54 | 10.24 | 9.84 | 7.85 | 1.28 | 0.79 | 0.97 | 0.09 |
| Agmatine | 1.26 | 1.16 | 1.12 | 1.16 | 0.15 | 0.84 | 0.66 | 0.67 |
| Cadaverine | 0.87 | 1.33 | 1.07 | 0.87 | 0.36 | 0.72 | 0.73 | 0.37 |
| Histamine | 0.40 | 0.48 | 0.90 | 0.31 | 0.17 | 0.16 | 0.35 | 0.06 |
| Phenylethylamine | 0.13 | 0.15 | 0.15 | 0.17 | 0.03 | 0.62 | 0.49 | 0.90 |
| Putrescine | 2.21 | 2.44 | 3.03 | 2.31 | 0.56 | 0.67 | 0.55 | 0.41 |
| Spermidine | 0.99 | 1.19 | 1.07 | 0.91 | 0.12 | 0.87 | 0.41 | 0.17 |
| Spermine | 0.53 | 1.06 | 1.03 | 0.76 | 0.22 | 0.55 | 0.65 | 0.08 |
| Tryptamine | 0.58 | 1.73 | 0.85 | 0.62 | 0.47 | 0.34 | 0.38 | 0.16 |
| Tyramine | 0.58 | 0.69 | 0.61 | 0.74 | 0.08 | 0.16 | 0.62 | 0.93 |
| Total monogenic amines[8] | 0.17 | 0.20 | 0.16 | 0.21 | 0.02 | 0.12 | 0.96 | 0.64 |
| Decylamine | 0.17 | 0.16 | 0.16 | 0.17 | 0.03 | 0.95 | 0.98 | 0.80 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.
[7] Total biogenic amines = agmatine + cadaverine + histamine + phenylethylamine + putrescine + spermidine + spermine + tryptamine + tyramine.
[8] Total monogenic amines = decylamine + ethylamine + *sec*/*tert* butylamine.

***Nutrient digestibilities.*** No differences ($P > 0.05$) in total tract macronutrient digestibilities were observed in dogs in Experiment 1 (**Table 12**). In Experiment 2, dogs fed LAC tended to have greater total tract DM ($P = 0.05$) and CP ($P = 0.08$) digestibilities compared with dogs fed microcrystalline cellulose.

## TABLE 9

*Fecal biogenic and monogenic amine concentrations for dogs of Experiment 2 fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| | *μmol/g dry matter* | | | | | | | |
| Total biogenic amines[7] | 22.42 | 13.62 | 13.97 | 14.49 | 6.16 | 0.51 | 0.55 | 0.46 |
| Agmatine | 1.49 | 2.02 | 1.62 | 1.59 | 0.19 | 0.21 | 0.44 | 0.16 |
| Cadaverine | 5.33 | 1.61 | 1.88 | 1.82 | 2.09 | 0.38 | 0.45 | 0.39 |
| Histamine | 0.50 | 0.48 | 0.60 | 0.37 | 0.15 | 0.40 | 0.98 | 0.49 |
| Phenylethylamine | 0.19 | 0.24 | 0.15 | 0.18 | 0.06 | 0.46 | 0.36 | 0.88 |
| Putrescine | 8.27 | 3.98 | 4.77 | 4.64 | 2.63 | 0.41 | 0.60 | 0.44 |
| Spermidine | 1.25 | 1.11 | 1.43 | 1.26 | 0.15 | 0.30 | 0.27 | 0.91 |
| Spermine | 0.75 | 1.05 | 0.60 | 0.79 | 0.17 | 0.18 | 0.26 | 0.73 |
| Tryptamine | 3.83 | 2.11 | 2.18 | 2.98 | 1.47 | 0.76 | 0.79 | 0.41 |
| Tyramine | 0.82 | 1.01 | 0.74 | 0.86 | 0.15 | 0.30 | 0.45 | 0.78 |
| Total monogenic amines[8] | 0.33 | 0.23 | 0.16 | 0.22 | 0.09 | 0.85 | 0.33 | 0.35 |
| Decylamine | 0.20 | 0.19 | 0.16 | 0.18 | 0.02 | 0.84 | 0.23 | 0.45 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.
[7] Total biogenic amines = agmatine + cadaverine + histamine + phenylethylamine + putrescine + spermidine + spermine + tryptamine + tyramine.
[8] Total monogenic amines = decylamine + ethylamine + *sec*/*tert* butylamine.

**TABLE 10**

*Fecal volatile sulfur compound concentrations for dogs of Experiment 1 fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values[3] | | |
|---|---|---|---|---|---|---|---|---|
| Compound/h of fermentation | Control | FOS | LAC | FOS/LAC | SEM[4] | Trt[5] | H[6] | Trt × H[7] |
| | *nmol/g dry matter* | | | | | | | |
| Hydrogen sulfide | | | | | | | | |
| 2 h | 382.64 | 372.65 | 411.21 | 333.11 | | | | |
| 4 h | 488.09 | 379.77 | 555.44 | 370.57 | | | | |
| 24 h | 521.44 | 374.79 | 723.87 | 497.45 | 28.03 | 0.02 | <0.01 | 0.21 |
| Methanethiol | | | | | | | | |
| 2 h | 442.34 | 446.53 | 446.35 | 416.34 | | | | |
| 4 h | 555.92 | 461.32 | 571.10 | 460.35 | | | | |
| 24 h | 566.41 | 512.78 | 791.76 | 588.34 | 19.30 | 0.07 | <0.01 | 0.22 |
| Dimethyl sulfide | | | | | | | | |
| 2 h | 4.67 | 4.86 | 5.04 | 3.56 | | | | |
| 4 h | 5.15 | 5.08 | 6.70 | 5.59 | | | | |
| 24 h | 7.45 | 11.25 | 6.96 | 15.85 | 0.71 | 0.96 | 0.01 | 0.19 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] One set of *P* values is given for each compound, which is to be used for all times and treatments.
[4] SEM = pooled SEM.
[5] Main effect of treatment.
[6] Main effect of hour.
[7] Interaction effect between treatment and hour.

## DISCUSSION

Depending on the prebiotic or probiotic used in an experiment, various outcomes may be observed because each possesses properties unique to itself. Therefore, it is important to distinguish which is used in each experiment. The prebiotic used in this study was a common short-chain FOS and has been used in numerous experiments in several species including dogs. Although our probiotic (*Lactobacillus acidophilus* NCFM) is commonly used in experimental studies, it is a

**TABLE 11**

*Fecal volatile sulfur compound concentrations for dogs of Experiment 2 fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values[3] | | |
|---|---|---|---|---|---|---|---|---|
| Compound/h of fermentation | Control | FOS | LAC | FOS/LAC | SEM[4] | Trt[5] | H[6] | Trt × H[7] |
| | *nmol/g dry matter* | | | | | | | |
| Hydrogen sulfide | | | | | | | | |
| 2 h | 2028.22 | 1627.76 | 1651.61 | 1456.36 | | | | |
| 4 h | 1965.23 | 1611.30 | 1860.58 | 1266.50 | | | | |
| 24 h | 889.30 | 960.62 | 1385.32 | 1349.89 | 173.97 | 0.98 | 0.04 | 0.50 |
| Methanethiol | | | | | | | | |
| 2 h | 1024.26 | 757.10 | 882.52 | 671.17 | | | | |
| 4 h | 930.14 | 640.06 | 859.87 | 697.63 | | | | |
| 24 h | 644.99 | 645.32 | 897.81 | 883.37 | 76.85 | 0.80 | 0.53 | 0.37 |
| Dimethyl sulfide | | | | | | | | |
| 2 h | 8.09 | 6.93 | 8.27 | 6.02 | | | | |
| 4 h | 10.87 | 8.36 | 9.57 | 7.83 | | | | |
| 24 h | 15.04 | 13.13 | 20.71 | 13.53 | 1.21 | 0.09 | <0.01 | 0.19 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] One set of *P*-values is given for each compound, which is to be used for all times and treatments.
[4] SEM = pooled SEM.
[5] Main effect of treatment.
[6] Main effect of hour.
[7] Interaction effect between treatment and hour.

**TABLE 12**

*Total tract nutrient digestibilities by dogs fed diets with or without fructooligosaccharides (FOS) and Lactobacillus acidophilus (LAC) for 28 d*[1]

| | Treatment[2] | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | FOS | LAC | FOS/LAC | SEM[3] | FOS[4] | LAC[5] | FOS × LAC[6] |
| | % | | | | | | | |
| Experiment 1 | | | | | | | | |
| Dry matter | 75.6 | 73.3 | 73.1 | 73.5 | 1.16 | 0.41 | 0.36 | 0.27 |
| Organic matter | 86.4 | 85.3 | 85.5 | 85.1 | 0.71 | 0.31 | 0.43 | 0.62 |
| Crude protein | 75.7 | 73.7 | 74.3 | 72.6 | 1.46 | 0.22 | 0.42 | 0.94 |
| Experiment 2 | | | | | | | | |
| Dry matter | 76.8 | 75.3 | 78.0 | 78.6 | 1.08 | 0.68 | 0.05 | 0.33 |
| Organic matter | 87.0 | 85.9 | 87.5 | 87.8 | 0.74 | 0.56 | 0.13 | 0.35 |
| Crude protein | 77.2 | 75.4 | 78.6 | 78.7 | 1.26 | 0.49 | 0.08 | 0.46 |

[1] Values are means, $n = 5$.
[2] Treatments include: Control, 2 g sucrose + 80 mg microcrystalline cellulose twice daily; FOS, 2 g FOS + 80 mg microcrystalline cellulose twice daily; LAC, 2 g sucrose + $1 \times 10^9$ LAC twice daily; FOS/LAC, 2 g FOS + $1 \times 10^9$ LAC twice daily.
[3] SEM = pooled SEM.
[4] Main effect of FOS.
[5] Main effect of LAC.
[6] Interaction effect between FOS and LAC.

human-derived strain. Ideally, the probiotic used would be derived from the same species to which it is being supplemented. However, to our knowledge, there are no canine-derived probiotics currently available for experimental use.

Probiotic consumption decreased ($P < 0.05$) food intake by dogs in Experiment 1. Although dogs were randomly allotted to treatments, those allotted to receive probiotics tended ($P = 0.06$) to be older than dogs allotted to the cellulose treatments in this experiment. Older animals have decreased energy requirements due to decreased basal metabolic rate and activity level (28). It is unknown whether the decrease in food consumption was due to probiotic supplementation, increased age or both. The decreased food intake by dogs supplemented with LAC resulted in decreased fecal output by these dogs. Probiotic supplementation had no effect on food intake in Experiment 2. Fructooligosaccharide supplementation did not affect food intake in either experiment. Dogs supplemented with FOS in Experiment 2 had lower ($P < 0.05$, g/d DMB; $P = 0.06$, g/d as-is) fecal output than dogs fed sucrose. This observation was surprising because FOS has been shown to increase wet fecal weight in previous experiments in dogs (29). Fructooligosaccharide supplementation altered concentrations of several microbes in Experiment 2. In addition to increasing sheer numbers of total aerobes, bifidobacteria and lactobacilli in the gut, FOS may have increased microbial activity, resulting in increased substrate degradation and lower fecal volume.

Fructooligosaccharide supplementation has been shown to increase bifidobacteria concentrations in several species, including dogs (9), mice (30) and humans (31). In the experiment of Russell (9), dogs fed either 1% FOS or 3% chicory had greater bifidobacteria concentrations than those fed a control diet. Dogs in our Experiment 2 had greater ($P < 0.05$) bifidobacteria concentrations than dogs fed sucrose even though FOS represented <1% of the dietary intake. Probiotic supplementation also had a positive effect on bifidobacteria populations. Dogs in Experiment 2 supplemented with LAC tended ($P = 0.08$) to have increased bifidobacteria concentrations compared with dogs fed cellulose. In an experiment using human subjects, supplementation of *Lactobacillus casei* strain Shirota increased ($P < 0.05$) fecal *Bifidobacterium* concentrations compared with control subjects after 2 wk, but not after 4 wk, on trial (32). Dogs in Experiment 2 fed FOS tended to have greater ($P = 0.08$) lactobacilli concentrations than dogs supplemented with sucrose. An increase in bifidobacteria often is accompanied by a decrease in clostridia concentrations (31). Depending on the strain of clostridia, this decrease may or may not be beneficial. In the current experiment, dogs in Experiment 1 fed FOS tended ($P = 0.08$) to have decreased *C. perfringens* concentrations, a positive indicator of colon health.

Concentrations of *E. coli*, one of the primary aerobic microbial species in the colon, were not influenced by treatment in the current experiment. However, total aerobe concentrations were increased ($P < 0.05$) in Experiment 2 as a result of FOS supplementation. Howard et al. (33) reported similar results in Beagles. Because specific aerobic species other than *E. coli* were not measured, it is unknown whether the increase in total aerobes was beneficial or harmful.

SCFA and in particular, butyrate, are the main energy source for colonocytes. Butyrate is the preferred energy substrate of colonic epithelium (34). Lactate is a major end product of the lactate-producing species, *Lactobacillus* and *Bifidobacterium*. An increased lactate concentration often decreases luminal pH and is a potent antimicrobial substance to several pathogenic species. Fructooligosaccharide supplementation also has been reported to increase butyrate concentrations in vitro (35) and in vivo (36,37). In the current experiment, dogs supplemented with FOS had greater fecal concentrations of lactate ($P < 0.05$, Experiment 1; $P = 0.06$, Experiment 2) and butyrate ($P = 0.06$, Experiment 1; $P < 0.05$, Experiment 2) than dogs fed sucrose. Although FOS supplementation clearly increased lactate production in these experiments, the increased butyrate seemed to be associated more with an overall increase in total SCFA rather than being a consequence of a specific increase in butyrate-producing bacteria.

The metabolism of protein in the colon by microflora may be modified by the availability of substrate, particularly by dietary carbohydrate (38,39). Fermentable carbohydrates, including FOS, may decrease the concentration of putrefactive compounds by providing gut microflora with an additional

energy supply. When energy (carbohydrate) supplies are limited, bacteria ferment amino acids (AA) to SCFA and ammonia to obtain energy (40). However, if a sufficient energy source is provided, the luminal concentrations of nitrogenous compounds decrease and the concentrations of fecal N (bacterial mass) increase (41,42).

In the current experiment, FOS supplementation decreased the concentrations of several putrefactive compounds present in feces. Isobutyrate, isovalerate and total BCFA concentrations were lower in dogs fed FOS in Experiment 2. In Experiment 1, supplementation of FOS + LAC decreased fecal concentrations of these same BCFA compared with FOS and LAC alone. Fructooligosaccharide supplementation also had a positive effect on fecal concentrations of aromatic AA catabolites (phenols and indoles). In Experiment 1, dogs fed FOS + LAC had lower fecal indole and total phenol and indole concentrations than dogs given FOS or LAC alone. Dogs fed FOS also had lower total phenol concentrations compared with dogs fed sucrose in this experiment. In Experiment 2, dogs fed FOS tended to have lower ($P = 0.07$) fecal indole concentrations than dogs fed sucrose. These results agree with a previous experiment done in our laboratory with dogs fed FOS (11) and research performed with cats and dogs in Japan using a similar nondigestible oligosaccharide, lactosucrose (43,44).

The gases in flatus are primarily N, $O_2$, $CO_2$, $H_2$ and $CH_4$ originating from swallowed air, diffusion from blood and bacterial fermentation; < 1% of the gas volume is responsible for malodor, with much of this amount attributed to sulfur gases produced by bacteria that use sulfate during oxidative reactions (45). Suarez et al. (46) reported that hydrogen sulfide, methanethiol and dimethyl sulfide were the primary sulfur gases measured in human flatus, with hydrogen sulfide as the predominant sulfur gas in 78% of the samples.

Because we were not able to directly collect rectal gas samples from dogs in the current experiment, we elected to measure volatile sulfur compound concentrations produced by fresh fecal samples that were incubated for 2, 4 and 24 h at 39°C. Nothing was added to the feces in the incubation syringes. Therefore, the volatile sulfur compounds measured in each syringe comprised the sum of gases present at the time of collection (sulfur compounds present in feces that moved into the gaseous phase), plus those produced after collection (microbial fermentation of any remaining substrate in the feces). In Experiment 1, treatment had an effect on the production of hydrogen sulfide and methanethiol, but not dimethyl sulfide. In Experiment 2, dimethyl sulfide tended to be influenced by treatment, whereas hydrogen sulfide and methanethiol were not. As time of fermentation increased, sulfur concentrations increased ($P < 0.05$) for all gases in both experiments, except for methanethiol in Experiment 2. No treatment × time interactions were observed for any volatile sulfur compound in either experiment. In Experiment 1, samples collected from dogs consuming SUC + LAC had the highest hydrogen sulfide and methanethiol concentrations. Similarly, fecal samples from dogs fed SUC + LAC in Experiment 2 had the highest dimethyl sulfide concentrations. It is unknown why samples from dogs fed SUC + LAC produced higher concentrations of sulfur gases.

Because availability of substrate may be a limiting factor in sulfur gas production, probiotic supplementation may have increased the amount of substrate available for sulfur-reducing bacteria. It is possible that LAC increased substrate by increasing mucin production and (or) shortening intestinal transit time, decreasing small intestinal digestibility of protein and increasing levels of undigested AA reaching the colon. Mack et al. (47) reported that incubation of *Lactobacillus plantarum* 299v with HT-29 intestinal epithelial cells increased MUC2 and MUC3 (intestinal mucin genes) mRNA expression levels. Bartram et al. (48) reported shorter ($P < 0.05$) mouth-to-cecum transit times in adult humans consuming yogurts enriched with *Bifidobacterium longum* and lactosucrose compared with subjects consuming conventional yogurts.

Although no differences in total tract nutrient digestibilities were observed among treatments in Experiment 1, dogs in Experiment 2 fed LAC tended to have greater total tract DM, OM and CP digestibilities than dogs fed cellulose. There are some reports in the literature regarding increased growth and feed conversion in poultry and swine from probiotic supplementation (49,50), but nothing has been reported in companion animals. In the current experiment, total tract digestibilities were measured. It is unknown whether the enhanced digestibility occurred in the upper part of the gastrointestinal tract or in the hindgut.

To conclude, supplementation of FOS positively influenced indices of gut health in the canine. Fructooligosaccharides enhanced gut microbial ecology by increasing concentrations of beneficial microbial populations (e.g., bifidobacteria, lactobacilli) and decreasing concentrations of potential pathogens (e.g., *C. perfringens*). Supplementation of FOS also enhanced indices of gut health by increasing fecal butyrate and lactate concentrations and decreasing several putrefactive compounds (e.g., BCFA, phenols, indoles) present in feces. It appears that LAC may enhance some beneficial microbial populations and increase total tract nutrient digestibility. However, LAC supplementation also may increase the concentration of potentially toxic volatile sulfur compounds found in feces. The supplementation of FOS and LAC together as a synbiotic may prove to be beneficial because it may decrease the concentration of several fecal putrefactive compounds (biogenic amines, BCFA, phenols, indoles) to a greater extent than either supplement consumed alone.

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